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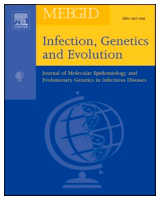

Research paper

Microevolution of a *Mycobacteroides abscessus* subsp. *bolletii* strain in a clinical persistent infection

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ABSTRACT

Mycobacteroides abscessus complex (MAB), a fast-growing nontuberculous mycobacterium, is emerging as a significant infectious disease threat, due to both intrinsic and acquired resistance mechanisms to antibiotics and disinfectants and the need for extensive and multidrug regimens for treatment. Despite the prolonged regimens, outcomes are poor and persistence cases have been reported. Here, we describe clinical, microbiologic and genomic features of a *M. abscessus* subsp. *bolletii* (*M. bolletii*) strain consecutively isolated from a patient within an eight-year infection period.

From April 2014 to September 2021, the National Reference Laboratory for Mycobacteria received eight strains isolated from a male patient. Species identification, molecular resistance profile and phenotypic drug susceptibility were determined. Five of these isolates were recovered for further in-depth genomic analysis.

Genomic analysis confirmed the multidrug resistant pattern of the strain and also other genetic changes associated with adaptation to environment and defence mechanisms. We highlight the identification of new mutations in locus MAB_1881c and in locus MAB_4099c (*mps1* gene), already described as associated with macrolides resistance and morphotype switching, respectively. Additionally, we also observed the emergence and fixation of a mutation in locus MAB_0364c that appeared at a frequency of 36% for the 2014 isolate, 57% for the 2015 isolate and 100% for the 2017 and 2021 isolates, clearly illustrating a fixation process underlying a microevolution of the MAB strain within the patient.

Altogether these results suggest that the observed genetic alterations are a reflection of the bacterial population's continuous adaptation and survival to the host environment during infection, contributing to persistence and treatment failure.

1. Introduction

The species *Mycobacteroides* (or *Mycobacterium*) *abscessus* (MAB) is a fast growing nontuberculous mycobacterium (NTM) known to be associated with an increasing number of disease cases worldwide (Johansen et al., 2020). It is particularly difficult to eradicate, due to both its intrinsic resistance mechanisms (i.e., innate, not acquired during the course of antibiotic exposure) to antibiotics and disinfectants and to the need for extensive and multidrug regimens for treatment. Although transmission routes are not yet well established and MAB infections are assumed to be acquired from the environment, recent studies demonstrate that most individuals are infected with one of several dominant circulating clones, which suggests a possible global transmission (Ruis et al., 2021; Diricks et al., 2022). How these clones emerged and spread throughout the globe is still unclear, and person-to-person transmission presumably through indirect mechanisms is a possibility (Ruis et al., 2021; Bryant et al., 2013; Aitken et al., 2012).

Genomic studies are contributing to a better understanding of this emerging species, mostly on its epidemiology, antimicrobial resistance and adaptation mechanisms. Moreover, these studies recently validated

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the subdivision of MAB complex into three subspecies: 1) *M. abscessus* subsp. *abscessus* (*M. abscessus*), the most common subspecies of the complex; 2) *M. abscessus* subsp. *massiliense* (*M. massiliense*) and 3) *M. abscessus* subsp. *bolletii* (*M. bolletii*) (Victoria et al., 2021). All of these subspecies are opportunistic pathogens and although genetically related, they show differences in intrinsic drug resistance, association with clinical disorders and mode of transmission (Tan et al., 2017; Lewin et al., 2021; Griffith, 2014). For example, *M. bolletii* is unfrequently isolated from clinical samples, but is often associated with multidrug resistance (Tan et al., 2017). Nevertheless, bacteria from the MAB complex are considered one of the most resistant mycobacteria, and the majority of the strains harbour a highly dynamic open pan-genome that may also contribute to their evolution and adaptation to stressful environments including within the host (Johansen et al., 2020; Victoria et al., 2021).

The manifestation of the disease is the reflex of complex interactions between host (immune status, genetic risk factors and prior lung disease), pathogen (pathogenicity and virulence) and environmental determinants such as the infecting dose and duration of exposure (Johansen et al., 2020). MAB often cause severe respiratory, skin, and mucosal infections and have recently emerged as major pathogens, greatly affecting people with cystic fibrosis (Griffith et al., 2007; Koh et al., 2017). In this context, a correct and early identification of the MAB subspecies, with clinical, radiological and laboratory findings is essential for the management of the disease. Since the symptoms are non-specific there is often a delay in diagnosis, resulting in disease progression and eventually leading to more complicated treatments. These regimens require the prolonged use of multiple drugs, which is costly and may cause severe adverse reactions for the patient as these drugs usually show high toxicity (Griffith et al., 2007; Koh et al., 2017). For MAB-complex associated infections, the American Thoracic Society has recommended an empiric regimen comprising a combination of a macrolide [clarithromycin (CLR) or azithromycin (AZM)], an aminoglycoside [amikacin (AMK)], and a β-lactam [cefoxitin (FOX) or imipenem], for a period of culture-free for more than one year (Griffith et al., 2007).

Although it is assumed that nearly 50% of the disease cases are successfully resolved (Johansen et al., 2020; Kreutzfeld et al., 2013), with the patient showing a clearance of both symptoms and microbiological isolations of the agent, cases of disease persistence have been reported (Lewin et al., 2021; Kreutzfeld et al., 2013; Harris et al., 2021; Esther et al., 2010). In the present study, we aim to describe clinical, microbiologic and genomic features of a *M. bolletii* strain consecutively isolated from a patient within an eight year infection period. The analysis of the long-term genomic evolution of this strain may contribute to a better understanding of the mechanisms underlying the persistence of these infections.

2. Materials and methods

2.1. Clinical case description

In the beginning of 2013 (Fig. 1), a 63 year old male patient attended to a hospital consultation presenting moderate respiratory symptoms that included cough, bloody sputum and atypical chest pain. Clinical history included a benign prostatic hyperplasia and pulmonary tuberculosis (PTB) at the age of 7. After imaging exams, he was referred with lower left lobe bronchiectasis. Bronchoalveolar lavage analysis from March 2013 revealed the presence of Alcohol-Acid Fast Bacilli, and the patient was clinically diagnosed with PTB and treated accordingly (i.e., two months of isoniazid PO 250 mg/day, rifampicin PO 500 mg/day pyrazinamide PO 1250 mg/day and ethambutol PO 750 mg/day – HRZE, followed by four months of isoniazid PO 250 mg/day and rifampicin PO 750 mg/day – HR). Of note, *M. tuberculosis* isolation in culture was never achieved.

After PTB treatment completion, in April 2014, a follow-up sputum sample was collected leading to the first isolation of *M. bolletii*. The worsening of the symptoms (fatigue, cough, purulent bloody sputum, atypical chest pain and bronchiectasis in the lower left and upper right lobes) led to hospitalization in November 2015 where he was first treated for a MAB infection (Fig. 1). The treatment, that included AMK IV 750 mg/day, AZM PO 500 mg/day, clofazimine (CFZ) PO 100 mg/day and FOX IV 12 g/day during hospitalization (two months) followed by six months of AZM PO 500 mg/day and CFZ PO 100 mg/day in ambulatory (Fig. 1), was completed after eight months with mild clinical improvement. From 2017 up to 2019, although no worsening of clinical symptoms was observed, the *M. bolletii* continued to be isolated and a new treatment was proposed but the patient declined it.

During 2021 the patient's symptoms worsened again leading to a bilateral pulmonary emphysema and a new hospitalization, in a respiratory isolation unit, in November. A second MAB-directed treatment was initiated (Fig. 1) including AMK IV 750 mg/day, tigecycline (TGC) IV 50 mg/day, linezolid (LZD) PO 600 mg/day and CFZ PO 100 mg/day during hospitalization (three months), and LZD PO 600 mg/day plus CFZ PO 100 mg/day for seven months in ambulatory. Since then, no other MAB strains were isolated, and in September 2022 the patient was considered cured and the treatment regimen was interrupted.

2.2. Microbiological characterization of MAB isolates

From April 2014 to September 2021, the National Reference Laboratory for Mycobacteria (NRL-TB) received eight strains isolated from this patient. Species identification and molecular resistance profile were determined using GenoType *Mycobacterium* CM® and GenoType NTM-DR® (Hain Lifescience). Drug susceptibility testing (DST) was performed according to the Clinical and Laboratory Standards Institute (The Clinical and Laboratory Standards Institute (CLSI, 2011). Minimum inhibitory concentrations (MICs) of AMK, FOX, ciprofloxacin (CIP), CLR,

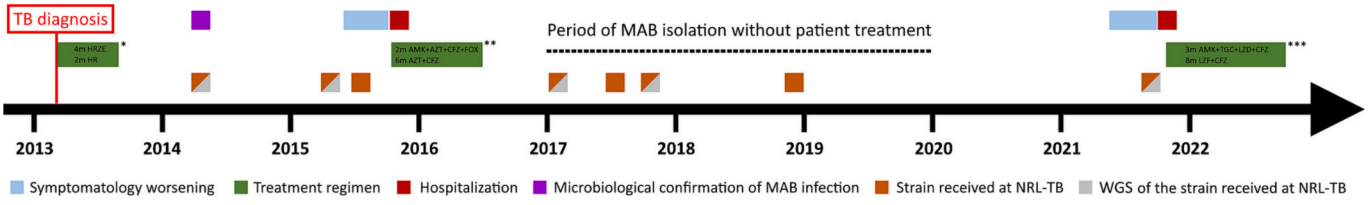

Fig. 1. Timeline of the persistent *Mycobacteroides abscessus* subsp. *bolletii* infection from 2013 to 2022. *Four months isoniazid PO (250 mg/day), rifampicin PO (500 mg/day), pyrazinamide PO (1250 mg/day), ethambutol PO (750 mg/day), followed by two months isoniazid PO (250 mg/day), rifampicin PO (500 mg/day); **Two months amikacin IV (750 mg/day), azithromycin PO (500 mg/day), clofazimine PO (100 mg/day), cefoxitin IV (12 g/day), followed by six months azithromycin PO (500 mg/day), clofazimine PO (100 mg/day); ***Three months amikacin IV (750 mg/day), tigecycline IV (50 mg/day), clofazimine PO (100 mg/day), linezolid PO (600 mg/day), followed by seven months linezolid PO (600 mg/day), clofazimine PO (100 mg/day). TB – tuberculosis; MAB – *Mycobacteroides abscessus* subsp. *bolletii*; WGS – Whole-genome sequencing; NRL-TB – National Reference Laboratory for Mycobacteria. HRZE – Isoniazid, rifampicin pyrazinamide and ethambutol; HR – Isoniazid and rifampicin; AMK – amikacin; AZT – azithromycin; CFZ – clofazimine; FOX – cefoxitin; TGC – tigecycline; LZD – linezolid.

DOX, LZD, moxifloxacin (MXF) and CFZ were determined (Supplementary Table S1), and *M. peregrinum* ATCC 700686 strain was used as the reference strain for quality control purposes. Briefly, the inoculums were prepared in cation-adjusted Mueller-Hinton broth, incubated at 30 °C and examined after 72 h. If visible growth in the control tube was observed, the MIC of each antibiotic was recorded and interpreted accordingly; otherwise the tubes were re-incubated and the readings repeated at day four or five. To ensure detection of inducible macrolide resistance, the incubation for CLR was maintained until day 14, unless resistance (MIC ≥8 μg/mL) was recognized earlier.

2.3. Whole genome sequencing and bioinformatics analysis

Five of the isolates collected from 2014 up to 2021 were recovered for further genomic analysis (of note, three of the strains were unrecoverable for further analysis). Genomic DNA was extracted as previously described (Somerville et al., 2005), and subjected to Nextera XT library preparation (Illumina, USA) prior to paired-end sequencing (2 × 250 bp or 2 × 150 bp) on either a MiSeq or a NextSeq 550 or 2000 instrument (Illumina, USA), according to the manufacturer's instructions. Genomes were assembled using INNUca v4.2.2 (https://github.com/B-UMMI/INNUca), an integrative bioinformatics pipeline for read quality analysis, species identification, and de novo genome assembly and improvement (Llarena et al., 2018) (see Table 1 for genome assembly details). Final draft genomes were annotated using Bakta v1.2.2 (Schwengers et al., 2021), using default parameters. Multi Locus Sequence Typing (MLST) was performed upon query and sequence submission to the PubMLST database (https://pubmlst.org/organisms/mycobacteroides-abscessus-complex).

To evaluate the genetic microevolution and adaptation of this strain within its host, quality processed reads were individually mapped against the annotated draft genome of the first isolated strain (Mabs_012014) and single nucleotide polymorphism (SNP) calling was performed using Snippy v4.5.1 (https://github.com/tseemann/snippy) (Snippy-Seemann, 2023), with a minimum per base coverage of 10 and minimum proportion of reads differing from the reference of 70%. As such, mutations found at 100% in frequency were defined as “fixed mutations”, and those found at a frequency below 70% as “emerging mutations”. A draft core-SNP-based phylogeny was constructed using parsnps v1.2 (Treangen et al., 2014), enrolling 114 publicly available *M. bolletii* genomes (see Supplementary Fig. S1 for details) and the GD91 genome as reference sequence (Genbank accession #CP065265.1). Whenever raw reads datasets were available, public genomes were also assembled using INNUca v4.2.2. For reference purposes, quality processed reads were also mapped to the reference genome ATCC 19977 (#CU458896.1), in order to report, when possible, the identified targeted regions relative to this reference, both for position and locus tag. Additionally, genomes were screened for known genetic markers associated with antimicrobial resistance (Johansen et al., 2020; Soroka et al., 2014; Halloum et al., 2017; Hurst-Hess et al., 2017; Pryjma et al., 2017).

3. Results

All isolates were identified as *M. bolletii* and belonging to a novel MLST profile, ST269. Clinical and microbiologic features of the isolates are summarized in Table 1. Molecular DST (mDST) using NTM-DR Kit allowed the detection of the *erm* (41) T28 type for all isolates, suggesting resistance to macrolides. Phenotypic DST (pDST), performed for four isolates (Mabs_012015, Mabs_012017, Mabs_032017 and Mabs_012021), revealed a consistent resistance pattern, with all of them being resistant to CLR, DOX, CIP and MXF and susceptible to LZD. Minor differences were observed for aminoglycosides resistance, as the strain isolated in 2021 was phenotypically (but not genetically) resistant to AMK. Additionally, we screened the sequenced genomes for already described specific genetic determinants known to confer resistance. All five isolates revealed the same mutation resistance pattern (see gDST in Table 1) except for novel mutations in MAB_1881c (encoding a TetR family transcriptional regulator). These alterations were only present in Mabs_012017 and Mabs_012021 isolates, namely a 12 bp deletion and a SNP leading to protein truncation, respectively (Supplementary Table S2). Of note, we observed that during 2017 the morphotype of the *M. bolletii* colonies switched from smooth to rough (Table 1).

The integration of the novel genome sequences with publicly available *M. bolletii* genomes revealed a high genetic diversity within the analysed dataset (Supplementary Fig. S1), with genomes differing by an overall mean pairwise distance of 17,285 SNPs in a total of 138,450 analysed variant sites (total core-genome analysed was 76% of the reference genome). In fact, novel *M. bolletii* genomes were genetically

Table 1
Clinical, microbiologic and genomic characterization of the strains received at National Reference Laboratory for Mycobacteria during 2014–2021.

	Mabs_012014	Mabs_012015	Mabs_022015	Mabs_012017	Mabs_022017	Mabs_032017	Mabs_012018	Mabs_012021
Year of isolation	2014	2015	2015	2017	2017	2017	2018	2021
Sample type	sputum	sputum	sputum	BAL	BAL	sputum	sputum	sputum
Colony morphotype	smooth	smooth	smooth	smooth	smooth	rough	rough	rough
mDST								
MA	R	R	R	R	R	R	R	R
AG	S	S	S	S	S	S	S	
pDST								
FOX	–	–	S	S	–	S	–	S
AMK	–	–	S	S	–	S	–	R
CLR	–	–	–	R(3)*	–	R(5)*	–	R(6)*
DOX	–	–	–	R	–	R	–	R
LZD	–	–	–	S	–	S	–	S
CIP	–	–	R	R	–	R	–	R
MXF	–	–	–	I	–	I	–	R
gDST	I303Q and L304M *embB* (MTB_Rv3795) for ethambutol; *erm(*41) T28 (MAB_2297) for macrolides; *gyrA*-Ala74 (MAB_0019) and *gyrB* Arg482 e Asn499 (MAB_006) for fluoroquinolones; presence of *arr_mab* gene (MAB_0591) for rifamycin group; presence of *bla_mab* gene (MAB_2875) for β-lactams; presence of Mab_ApH (MAB_2385) for streptomycin; presence of MabTetX (MAB_1496c) enzyme for tetracycline.							
Genome statistics								
Depth of coverage	72.49	78.14	–	118.08	–	82.54	–	88.04
# of contigs	132	84	–	70	–	64	–	37
Genome size (bp)	4,873,488	4,857,614	–	4,859,582	–	4,857,984	–	4,859,417
ENA accession	ERR10554471	ERR10554469		ERR10554472		ERR10554468		ERR10554470

R – resistant; S – sensitive; I – intermediate resistance; mDST – molecular drug susceptibility testing- Hain Lifescience®; pDST – phenotypic drug susceptibility testing; gDST – genomic determinants of resistance present in all strains; MA – macrolides; AG – aminoglycosides; FOX – cefoxitin; AMK – amykacin; CLR – clarithromycin; DOX – doxycycline; LZD – linezolid; CIP – ciprofloxacin; MXF – moxifloxacin; BAL – bronchoalveolar lavage; ENA – European Nucleotide Archive. *number of days of incubation until macrolides resistance recognition (MIC >8 μg/mL).

distant from those that are publicly available, with the closest genome (ERR374175) differing by 13,083 SNPs, in the applied analysis. In-depth genomic analysis of the novel *M. bolletii* genomes revealed a strong similarity among them (Supplementary Fig. S1), with a discreet emergence of mutations from 2014 up to 2021, indicating that the prolonged infection was caused by a single evolving strain (Fig. 2). The isolate collected in 2021 (Mabs_012021) was distinguishable from the 2014 isolate (Mabs_012014) by 6 indels and 11 SNPs. The majority of the SNPs (i.e., 8 out of the 11) corresponded to non-synonymous mutations and one of them was found to be fixed (i.e., 100% frequency) in all isolates from 2017 onwards (MAB_0364c, an ATPase similar to badF/badG/bcrA/bcrD type), but also in both isolates from 2014 and 2015, at frequencies of 35.7% and 57.4% respectively (Fig. 2), suggesting a microevolution of the MAB strain within the patient. Furthermore, we observed three distinct genetic alterations, including a large 37 bp deletion and 1 bp indels targeting an homopolymeric tract, each in a different isolate (Mabs_012015, Mabs_032017, Mabs_012021), in gene MAB_4099c, a probable non–ribosomal peptide synthetase (*mps1* gene). Still, most identified mutations targeted genes involved in drug resistance, defence mechanisms and adaptation to starvation and oxidative stress (Fig. 2, Supplementary Table S2).

4. Discussion

Mycobacteroides abscessus infections are a growing problem worldwide and despite prolonged multidrug treatment regimens, treatment outcomes are generally poor, and low conversion rates are reported (Johansen et al., 2020; Griffith et al., 2007; Koh et al., 2017). In Portugal, MAB is responsible for near 10% of NTM infections (Santos et al., 2022) and little is known about the clinical outcomes, recurrence or persistent rates. Here we describe one case of a chronically infected patient, enrolling the evolution of the infecting strain for over eight years (Fig. 1). During the infection period, the patient received two MAB-directed treatments and, although both regimens included the recommended antibiotics, the species *M. bolletii* is resistant to macrolides and treatments stopped before the one-year-treatment regimen recommended (Griffith et al., 2007; Koh et al., 2017). The genomic analysis of the multiple isolates confirmed the multidrug resistant pattern of the strain. Regarding macrolide resistance, besides the known *erm* (T28) type, we also observed two novel alterations in MAB_1881c (encoding a TetR family transcriptional regulator), an already described CLR resistance genetic target (Li et al., 2020). Nevertheless, as *M. bolletii* strain are resistant to macrolides due to *erm* gene (Johansen et al., 2020), the effect of these MAB_1881c alteration would not be expressed phenotypically. Still, their emergence reflect bacterial host adaptation, either due to continued macrolide exposure during treatment or as a consequence of a persistent infection as previously described (Bryant et al., 2021). Moreover, it allowed the identification of a more complete antibiotic susceptibility profile, even for antibiotics that are not usually phenotypically tested. All isolates presented mutations in genes conferring resistance to ethambutol, streptomycin, tetracyclines, fluoroquinolones, rifamycins and β-lactams (Table 1). The exception was observed for AMK, as the 2021 isolate appeared as phenotypically resistant. Although the search for already described mutation markers (Hurst-Hess et al., 2017; Pryjma et al., 2017; Rominski et al., 2017; Prammananan et al., 1998) was not successful, we cannot discard that this phenotype may be provided by a new, not yet described resistance mechanism. As the majority of the MAB treatments are initiated empirically, with the American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) recommending multidrug macrolide-based therapy (Griffith et al., 2007), inducible macrolide and aminoglycoside resistance is a reality and further complicates treatment

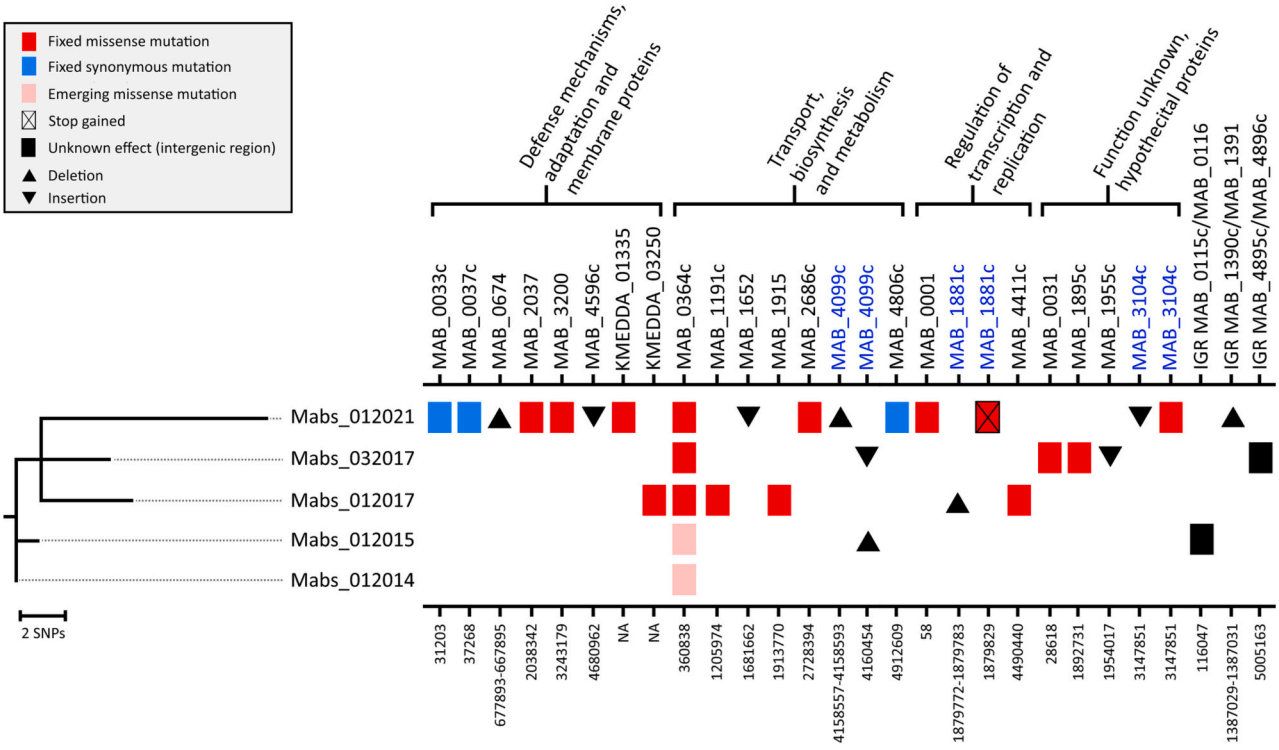

Fig. 2. Phylogeny of the persistent infection-causing *Mycobacteroides abscessus* subsp. *bolletii* isolates and detailed within patient mutational dynamics. Fixed mutations refer to single nucleotide polymorphisms (SNPs) or indels observed with a frequency ≥ 70%. Emerging mutations refer to SNPs or indels observed with a frequency < 70%. Gene designations (upper text) refer to the locus tags of the *M. abscessus* ATCC 19977 reference genome (#CU458896.1), with the exception of KMEDDA_01335 and KMDDA_03250 which refer to the draft annotation of Mabs_012014. Lower text refers to the SNP position in *M. abscessus* ATCC 19977. Genes targeted by mutations more than once are highlighted in blue. IGR – Intergenic region. NA – Not applicable. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Johansen et al., 2020; Pryjma et al., 2017). As such, a genomic approach, which allows a complete genetic resistance profile of the strain causing the infection, may enable a more patient-oriented treatment with better outcomes.

During the eight years of disease evolution, and despite clinical follow-up, the patient refused therapy for about four years (2017–2021), even when maintaining respiratory symptoms and the laboratory isolations of the infecting strain. Thus, it is likely that, in this period, optimal conditions were created for the bacteria to evolve and adapt, which was observed both phenotypically and genetically. In fact, not only did the infecting strain acquire a rough morphotype at the end of 2017, which is believed to be associated with a more virulent phenotype (Table 1, Fig. 2) (Victoria et al., 2021; Lewin et al., 2021; Pawlik et al., 2013; Ryan and Byrd, 2018), but also resistance to antibiotics increased from this point on (to AMK and MXF) as well as the emergence of mutations. We observed three distinct mutations in locus MAB_4099c (Fig. 2), a probable non–ribossomal peptide synthethase (*mps1* gene). Alterations in *mps1* have already been described as being implicated in morphotype switching (Johansen et al., 2020; Pawlik et al., 2013). A detailed genetic analysis of this locus revealed a 1 bp deletion and 1 bp insertion in a poly(G) tract for the 2015 and the last 2017 isolate, respectively, and a truncating 37 deletion for the 2021 isolate. Of note, the insertion observed in the 2015 isolate did not cause any visible phenotypical alteration. However, considering that poly(G) tracts are frequently associated with phase variation mechanisms underlying the ON/OFF state of proteins (Davidsen and Tønjum, 2006; Cayrou et al., 2021; Orsi et al., 2010; Esson et al., 2016), it is reasonable to speculate that the switch from the smooth to the rough colony morphotype here observed, may rely on the production/activity of the non–ribossomal peptide synthetase, as the three described genetic alterations may hypothetically underlie this enzymatic state. Nevertheless, although the identified alterations are likely triggering morphotype switching, as previously described in this genetic region (Johansen et al., 2020; Pawlik et al., 2013), its biological effect should undergo experimental confirmation. In fact, to date, only genetic changes in the locus *gpl*, that encodes proteins involved in synthesis or export of Glico-Peptido-Lipids, were confirmed as responsible for morphotype switching. One of these mutations was observed in MAB_4099c gene, namely an insertion of GC in 5′ region that is responsible for arrest of the whole operon and phenotypical alteration from smooth to rough (Johansen et al., 2020; Pawlik et al., 2013).

Most of the identified mutations targeted genes involved in drug resistance, defence mechanisms and adaptation to starvation and oxidative stress (Fig. 2, Supplementary Table S2). The comparative genome analysis of five isolates identified 25 genes with genetic alterations, the vast majority of them appearing in the 2017 and the 2021 isolates, with special emphasis in the latter. This enabled us to chronologically identify the emergence and fixation of genetic alterations in the genome of the infecting strain during the persistent infection (Fig. 2), likely illustrating an evolutionary adaptation scenario. Curiously, we could observe the chronological evolutionary emergence and fixation of one of these mutations in the locus MAB_0364c. In fact, this SNP (Supplementary Table 2) was identified at a frequency of 36% for the 2014 isolate, 57% for the 2015 isolate and 100% for the 2017 and 2021 isolates clearly illustrating a fixation process (Fig. 2) underlying a microevolution of the MAB strain within the patient. MAB_0364c encodes an ATPase similar to badF/badG/bcrA/bcrD type that was already described in other bacteria, more particularly environmental bacteria/opportunistic pathogens (Winsor et al., 2008) as being involved in Benzoyl-CoA pathway, which is an intermediate in the anaerobic metabolism of aromatic compounds, allowing survival and adaptation to low oxygen environments (Breese et al., 1998; Egland et al., 1997).

Altogether, these results suggest that the observed genetic alterations are a reflection of the bacterial population's continuous adaptation and survival to the host environment during its persisting infection. This follows previous observation that within-host evolution of *M. abscessus*, influenced by chronic infection, drives pathogenic adaptation (Bryant et al., 2021). Still, there are two points that may constitute a limitation of our study, namely: we did not have access to the patient's original biological samples (i.e., prior to strain isolation) and strain isolation with subsequent laboratory propagations may have not allowed the authentic representation of the whole MAB population infecting the patient; and the laboratory loss of three strains, which hampered the complete scenario of this strain's evolution during the persistent infection. Ultimately, we highlight the need for similar studies for a better understanding of the mechanisms involved in adaptation and survival of this challenging bacteria in the context of the human-pathogen arms race.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2023.105437.

Summary

Microevolution of a *M. bolletii* strain consecutively isolated from a patient in an eight-year infection period, is a reflection of the bacterial population's continuous adaptation and survival, contributing to persistence and treatment failure.

Credit author statement

I Rita Macedo, the corresponding author of this manuscript, certify that the contributors' and conflicts of interest statements included in this paper are correct and have been approved by all co-authors.

Data availability

All reads generated for the present study were deposited in the European Nucleotide Archive under the study accession number PRJEB57933 (individual run accession numbers are detailed in Table 1). Annotated genome sequence of *M. bolletii* isolate Mabs_012014 is available at: https://zenodo.org/record/7381908#.Y4dStnbP0uU.

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